from the column substantially all of said substance being determined which has not become bound.

9. A method as in claim 1 wherein said labeled form is of said substance being determined and wherein step (a) is accomplished by:

(a)(1) contacting said matrix with a predetermined quantity of said reference sample, and

(a)(2) thereafter contacting said matrix with a predetermined quantity of said liquid sample, the amount of said labeled component in said predetermined quantity of reference sample contacted with said matrix in step (a)(1) being in excess of that capable of binding with the specific binding partners immobilized with the matrix in the time that the predetermined quantity of reference sample and the matrix are in contact prior to step (a)(2), and the amount of said substance being determined in said predetermined quantity of liquid sample contacted with said matrix in step (a)(2) being sufficient to displace a portion of the labeled component bound to the specific binding partners immobilized with said matrix in the time that said predetermined quantity of liquid sample and said matrix are in contact prior to step (b).

10. A method as in claim 9 wherein said contact between said matrix and said predetermined quantity of liquid sample and said contact between said matrix and said predetermined quantity of reference sample are prolonged for predetermined incubation periods which may be the same or different.

11. A method as in claim 10 wherein said predetermined incubation periods range between 15 minutes and 12 hours.

12. A method as in claim 1 wherein said labeled form is of a specific binding partner to said substance being determined and wherein step (a) is accomplished by:

(a)(1) contacting said matrix with a predetermined quantity of said liquid sample, and

(a)(2) thereafter contacting said matrix with a predetermined quantity of said reference sample, the amount of specific binding partners immobilized with the matrix being in excess of that capable of binding with the total amount of the substance to be determined in said predetermined quantity to liquid sample contacted with said matrix in step (a)(1) in the time that the predetermined quantity of liquid sample and the matrix are in contact prior to step (a)(2), and the amount of said labeled component in said predetermined quantity of reference sample contacted with said matrix in step (a)(2) being sufficient to bind a portion or all of said substance being determined bound to said immobilized specific binding partners in the time that said predetermined quantity of reference sample and said matrix are in contact prior to step (b).

13. A method as in claim 1 which comprises the additional step of contacting said matrix with a liquid capable of equilibrating the pH of the matrix prior to step (a).

14. A method as in claim 13 wherein said equilibrating liquid comprises a buffer.

15. A method as in claim 1 wherein said eluting liquid capable of eluting from the column substantially all of the remaining unbound labeled component originating from the reference sample comprises a buffer.

16. A method as in claim 1 wherein said labeled component in said reference sample is radioactively labeled.

17. A method as in claim 16 wherein said radiocative label comprises a radioactive isotope of iodine.

18. A method as in claim 16 wherein step (c) includes measuring the amount of radioactivity emanating from said column, said amount being a function of the amount of said substance being determined in said liquid sample.

19. A method as in claim 16 wherein step (c) includes measuring the amount of radioactivity in the eluate resulting from step (b), said amount being a function of the amount of said substance being determined liquid in said sample.

20. A method as in claim 1 wherein said labeled form is labeled through the coupling of one of the components comprising an enzyme labeling pair of said substance being determined wherein an enzyme labeling pair comprises an enzyme and a substrate.

21. A method as in claim 20 wherein step (c) includes performing an enzymatic assay on the column by:

1. contacting said matrix with a fluid containing the other component comprising said enzyme labeling pair;
2. washing said column to remove substantially all of said component which reacted enzymatically, and
3. determining the amount of said other component which reacted enzymatically and which was washed from said column.

22. A method as in claim 20 wherein step (c) includes performing an enzymatic assay on the eluate resulting from step (b) by:

1. contacting said eluate with other component comprising said enzyme labeling pair, and
2. determining the amount of said other component which reacted enzymatically.

23. A method as in claim 1 wherein said substance to be determined is one of a specific binding pair of substances selected from the group consisting of antigens and their antibodies, haptens and their antibodies, enzymes and their substrates, hormones and their receptors, and vitamins and their receptors, and wherein said specific binding partner is the other comprising said specific binding pair of substances.

24. A method as in claim 23 wherein said substance being determined is selected from the group consisting of insulin, human placental lactogen, human chorionic gonadotropin, cholesterol, the vitamin B group, estrogens, digoxin, and digitoxin.

25. A method as in claim 1 wherein said matrix is made of a material comprising a polymeric substance.

26. A method as in claim 1 wherein said immobilized specific binding partner is chemically bound to said matrix.

27. A method as in claim 26 wherein said matrix is made of a material comprising a polymeric substance.

28. A method as in claim 27 wherein said polymeric substance contains a chemical group in its molecular structure selected from the group consisting of hydroxyl, primary amino and secondary amino groups.

29. A method as in claim 26 wherein said immobilized specific binding partner is chemically bound to said matrix through a coupling agent.

30. A method as in claim 29 wherein said coupling agent is selected from the group consisting of the cyanogen halides, the inorganic and organic cyanates, and the epihalohydrins.

31. A method as in claim 30 wherein said matrix comprises agarose.

32. A method as in claim 1 wherein said liquid sample is selected from the group consisting of body fluids and tissue extracts.

33. A method as in claim 1 wherein said liquid sample is serum.

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